Observation of Strain-Induced β Form in Poly(β-hydroxyalkanoates)

Recently, it has been shown that the reversible transformation of helical macromolecules to a planar zigzag conformation occurs in oriented crystalline fiber systems.[1-8] The classic example of this α–β transition is steam-stretched wool: upon drawing of the fiber to several hundred percent elongation, the α-helical form of this polypeptide is transformed to extended chains of β sheets.[5] In an example involving a synthetic polymer originally described by Knobloch and Statton,[4] the α–β transition was proposed as being responsible for the reversible elasticity at small extensions of oriented fibers of poly(pivalolactone) (PPL). In contrast to an α-phase transition for PPL, Clark[3] proposed that the *noncrystalline* domains were transformed to the extended chain conformation during cold stretching, as shown in Figure 1. Their deformation was responsible for the high initial modulus and the elastic behavior of PPL fibers for extensions of 2–5%. The strain-induced β phase was also observed for the parent polymer in the PPL series, poly(β-propiolactone),[6] as well as for the disubstituted polyester derived from α-methyl-α-*n*-propyl-β-lactone.[7]

These examples serve to demonstrate the importance of conformational transformations at the molecular level in providing unique and reversible mechanical properties of fibers along the lines of the "molecular spring" concept. With the present paper, we complement the PPL case using the biological poly(β-hydroxybutyrate-*co*-β-hydroxyvalerate), or PHB/V system (PHB/V has the same backbone as PPL but differs in that its single side-group methyl and/or ethyl is on the β carbon). Our results support the brief report by Yokouchi et al.[9] of a β phase for bacterial PHB homopolymer and extend these observations to the PHB/V copolymer system. Synthetic PHB is also shown to demonstrate a strain-induced β transition. Furthermore, an energy-minimized conformation for the β form is reported.

Cold Stretching of PHB/V. Bacterially produced PHB/V from industrial-scale batch cultures of *Alcaligenes eutrophus* was provided by Imperial Chemical Industries, U.K. A copolymer composition of 21% HV molar content was chosen for study due to its good film-drawing characteristics. As noted previously,[10,12] bacterial PHB/V is isotactic, exhibits a high molecular weight (approximately 200 000), and has a random distribution of hydroxyvalerate (HV) counits. It is well established that the mechanical properties of PHB/V in the 15–25% valerate composition range are improved in terms of flexibility and energy to break compared to pure PHB.[12,13] This "toughness" is attributable to the presence of dislocations, crystal strain, and smaller crystallites due to the insertion of the valerate unit into the PHB lattice. These effects make it much easier to cold stretch the PHB/V sample compared to pure PHB. Since films produced from the 21% HV sample exhibit lower moduli than PHB, they deform more easily to produce a β phase. In other words, for small deformations, the energy for deformation is directly related to the modulus and will thus be lower as the valerate composition is increased.

Uniform translucent films were prepared for drawing by dissolving PHB/V in 1,1,2,2-tetrachloroethane (5% solution) at room temperature and allowing these solutions to dry on a glass plate. Films subsequently placed in an oven at 140 °C for 3–5 min were cold-drawn within 1–5 h to approximately 800% by using either a hand-held mechanical stretcher or loosely hung weights.

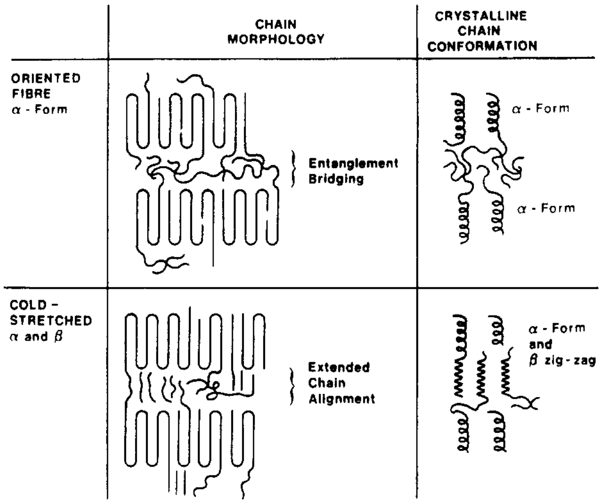

Figure 1. Schematic representation of the chain morphology and chain conformation of PHB before and after cold stretching. The original sample has only helical α-form chains, while in stretched films both helical and extended conformations are present. The β-form chains emanate from the noncrystalline domains.

X-ray fiber diffraction patterns of films containing the β form were recorded with the sample under tension by using an evacuated Warhus flat film camera mounted on a Philips 1730 X-ray generator, which provided Cu Kα X-radiation (λ = 0.1542 nm). Thermal analysis measurements were made on a Perkin-Elmer DSC-2 at a heating rate of 20 °C/min.

Previous publications[11,13] report that the X-ray diffraction pattern observed from powder samples of PHB/V copolymers containing up to 40 mol % hydroxyvalerate is that of PHB. Cold drawing of PHB/V films resulted in reflections on the fiber diffractogram (Figure 2a), which are not expected from the previously determined structure of PHB involving helical chains (the α form). These reflections are also not attributable to the PHV crystalline structure. In the diffractograms from cold-drawn films, one clearly sees the presence of a layer line streak at 0.460 nm (labeled S) just beyond the well-defined first layer line, as well as a new intense and broad equatorial reflection (labeled E) at 0.480 nm. The presence of these new reflections is interpreted as being due to the introduction of a β form, i.e., highly extended PHB/V chains. Packing in a hexagonal unit cell is likely since there is only a single new reflection on the equator. The failure of the chains to provide discrete diffraction spots, but rather a streak on their first layer line, is commonly explained by random translations of polymer chains in the fiber direction. A further possibility is that put forth by Clark[3] in his proposed structure for the β phase of poly(pivalolactone); not only may there be random translations in the fiber direction but also there may be random up-down chain orientations.

Of particular note is that the β-crystalline structure can be formed without first aligning the α-form lamellae. This is dependent on the "age" of the film as well as its valerate composition (i.e., its flexibility). Stretching of unaligned films that have been allowed to "set" for several weeks results in formation of an aligned β phase, without ordering the α phase. Presence of a β phase is indicated by the reflection labeled E in Figure 2b. This suggests that under these conditions the mechanism of β-phase formation in PHB/V is not via alignment and transformation of the α phase. Rather, the "α to β transition" emanates from

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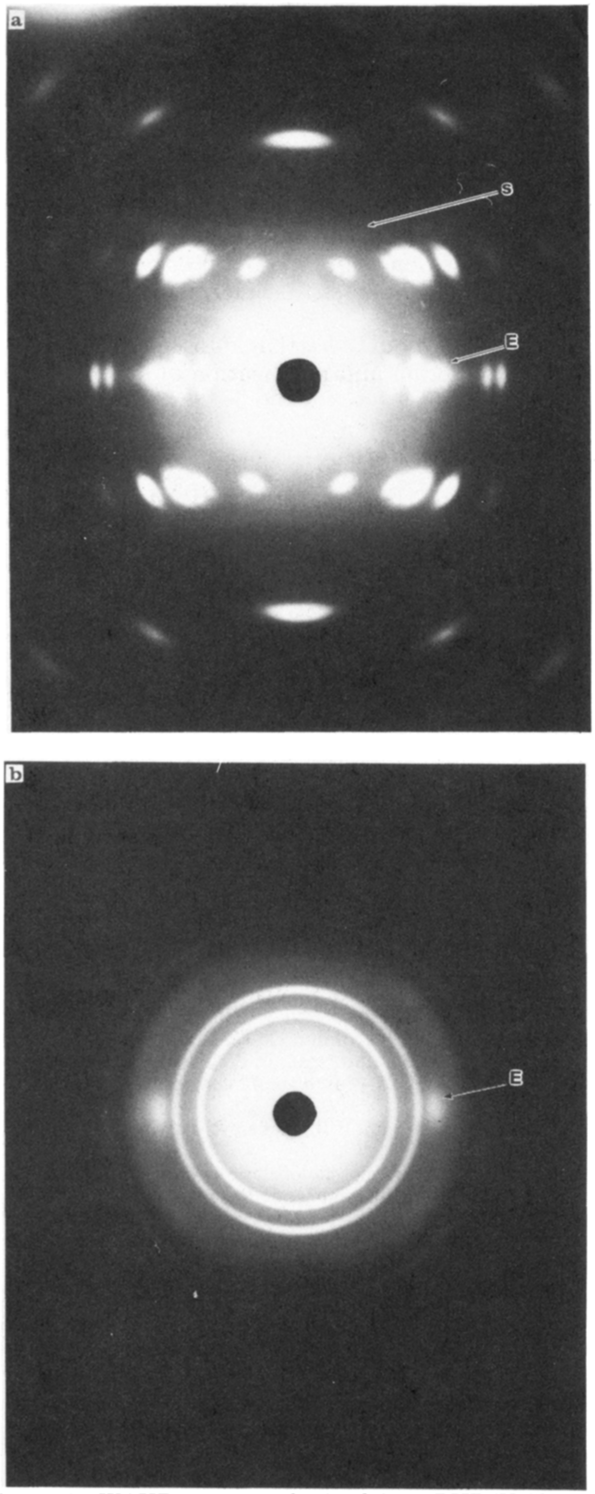

Figure 2. WAXD patterns obtained from PHB/V films: (a) stretched from films which were previously oriented in the α phase and (b) stretched from unoriented films (fiber axis is vertical). The intense equatorial reflection (E) and the first layer line streak (S) are indicative of the β conformation.

the amorphous areas between crystallites and is therefore not really a transition, but formation of a β form.

The rise per residue derived from the layer line streak in Figure 2a corresponds to 0.460 nm. This is less than the fiber repeat of 0.474 nm calculated for chains in the fully extended state[1] but much longer than the 0.298 nm observed for the α form. This rise per residue could result from a regular, nearly planar zigzag, conformation such as shown in Figure 3.

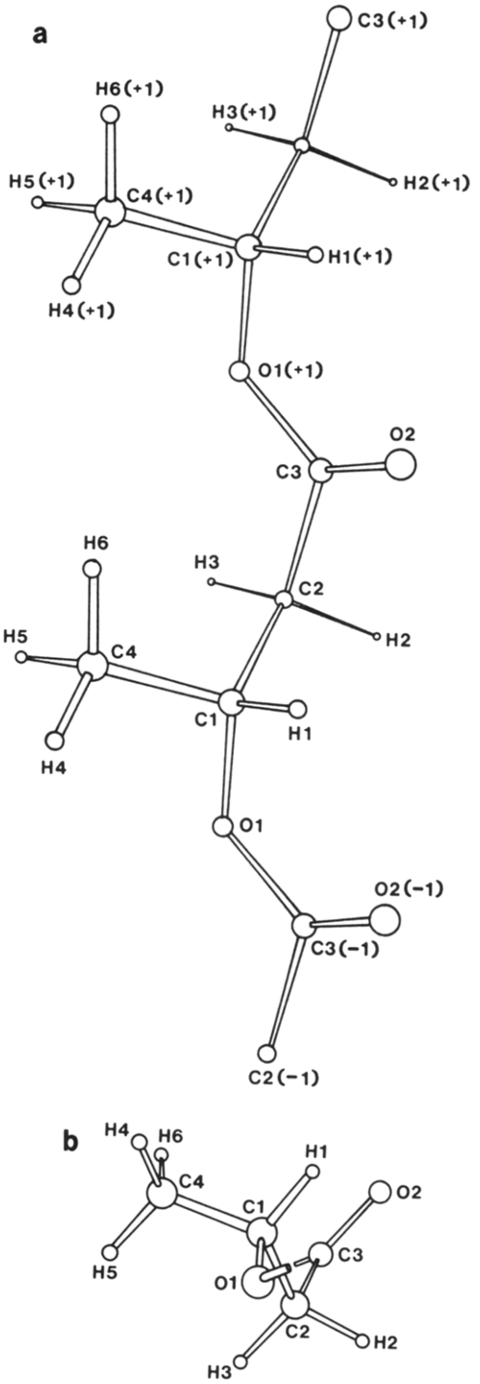

Figure 3. Proposed minimum-energy chain conformation of the β form of PHB viewed (a) perpendicular to the chain axis and (b) down the chain axis.

A simple conformational analysis based on energy minimization of a single PHB chain was carried out by using the observed fiber repeat. Simulations were performed on a Silicon Graphics Personal Iris workstation using POLYGRAF2.1 software[14] and the MM2 force field. The refined α-form conformation determined in this study is similar to that found in previous structural studies.[9,15] The refined conformation for the β form is shown in Figure 3 (bond lengths, bond angles, and torsion angles for both the α and β phases are presented in Table I). This conformation was arrived at by starting with the α-form bond lengths and angles[9,15] and constraining the rise per residue to 0.460 nm during energy minimization. In the β form, three of the four torsion angles in the backbone have changed appreciably from those found in the α form; only the C2–C3–O1(+1)–C1(+1) torsion angle remained near the trans position, as expected for bonds with partial double-bond character. Note that the torsion angles along

Table I
Bond Lengths, Bond Angles, and Torsion Angles for α and β Forms of Poly(β-hydroxybutyrate)

	bond length	
bond	α-form	β-form
C1–C2	1.54	1.54
C2–C3	1.52	1.52
C3–O1	1.35	1.35
O1–C1	1.41	1.42
C1–C4	1.54	1.54
C3–O2	1.21	1.21

	torsion angle	
angle	α-form	β-form
O1-C1-C2-C3	−51.2	−168.9
C1-C2-C3-O1	−46.1	−113.2
C2-C3-O1-C1	−168.2	172.1
C3-O1-C1-C2	156.5	112.6
C4-C1-C2-C3	−171.1	72.2
C1-C2-C3-O2	146.2	58.0
O2-C3-O1-C1	−0.7	1.2
C4-C1-O1-C3	−82.1	−126.2

	bond angle	
angle	α-form	β-form
C1-C2-C3	114.3	112.2
C2-C3-O1	109.6	110.5
C3-O1-C1	118.0	118.7
C4-C1-C2	111.6	111.6
O1-C1-C4	109.2	107.9
C2-C3-O2	123.8	123.2
O2-C3-O1	125.4	125.7
O1-C1-C2	107.8	108.8

the chain are not all-trans nor is the chain fully extended. For example, the angle between the methyl (C1–C4) and carbonyl (C3–O2) bond vectors, which should be 60° for an all-trans conformation, is calculated to be 100°. The methyl and carbonyl groups twist away from each other in order to minimize the van der Waals interactions between them. This arrangement of the ester dipoles would result in a net dipole moment for the β form, which is different from that of the α form. Since the piezoelectric properties of PHB/V, which have been reported previously,[16,17] depend on the ester dipole arrangement, such variations would affect this phenomenon.

Cold Stretching of Low-Tacticity PHB. A synthetic PHB sample was prepared by using a procedure similar to that described previously by Bloembergen et al.[18] By careful adjustment of experimental conditions and subsequent purification, a sample containing 64% isotactic diads and a molecular weight of 278 000 was obtained. This material was cast into translucent, tough films, which were cold-stretched 500% in a similar fashion to the procedure outlined above for the bacterial samples. When the sample was removed from the stretching device, it contracted to approximately one-third of its extended length and could then be reversibly drawn to the initial draw ratio.

The modulus of this material was not as high as that of the totally isotactic bacterial PHB. The layer line diffraction streak and equatorial reflection, indicative of the β form, were found at identical locations on the X-ray diffractograms obtained from both synthetic PHB (64% isotactic) and bacterial PHB (100% isotactic).

Conclusions. As discussed above, there are two distinctly different ways to improve the "toughness" of PHB; either to form its valerate copolymers by bacterial synthesis or to disrupt crystallinity by decreasing stereoregularity (as is the case for synthetic PHB samples). Upon cold drawing, both types of polymers "store" the energy of deformation in a metastable β phase. The formation of a β-crystalline phase, though, is not necessarily due to an α to β transition as in PPL but may be due to strain-induced crystallization of the amorphous regions.

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